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GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION
FEDERAL GRAIN INSPECTION SERVICE
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CHAPTER 12

RIDASCREEN® FAST AFLATOXIN SC TEST KIT

12.1 GENERAL INFORMATION

The RIDASCREEN® FAST Aflatoxin SC test is a competitive enzyme immunoassay for the quantitative analysis of aflatoxin in select grains and commodities. **The test kit is limited to providing aflatoxin measurements between 5 – 100 ppb.**

12.2 PREPARATION OF SOLUTIONS

a. Extraction Solution.

The extraction solvent used in the RIDASCREEN® FAST Aflatoxin SC test is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (ACS grade or better) and 30 percent water.

(1) Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.

(2) Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

(3) Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.

(4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

b. Wash Solution.

(1) Dissolve the contents of the packet containing the buffer salt in 1 liter of distilled water.

(2) Swirl to mix.

(3) Store this solution in a refrigerator until needed. The solution expires 4 weeks after preparation.

12.3 EXTRACTION PROCEDURES

a. Extraction procedure for Corn, Corn Meal, Corn Flour, Wheat, Cracked Corn, Corn Soy Blend, Popcorn, Corn Gluten Meal, Soybeans, Sorghum, Corn Germ Meal

(1) Transfer 50 grams of ground sample into an extraction mixing jar.

(2) Add 250 ml of the (70/30) methanol/water extraction solvent.

(3) Cover the extraction jar and blend on high speed for 2 minutes.

(4) Filter approximately 1.5 ml of the extract through a filtering syringe or equivalent.

(5) Dilute 1 ml of the filtrate with 1 ml of distilled or deionized water.

(6) Proceed to test procedures.

b. Extraction procedure for distillers dried grains w/ solubles (DDGS)

(1) Transfer 50 grams of ground sample into an extraction mixing jar.

(2) Add 250 ml of the (70/30) methanol/water extraction solvent.

(3) Cover the extraction jar and blend on high speed for 2 minutes.

(4) Add 50 g of the stabilization buffer (P4541) to the extraction jar and blend for an additional 30 seconds.

(5) Filter approximately 1.5 ml of the extract through a filtering syringe or equivalent.

(6) Dilute 1 ml of the filtrate with 2 ml of distilled or deionized water.

(7) Proceed to test procedures.

NOTE: This increases the dilution ratio and the results for DDGS must be multiplied by a factor of 1.5. For example if the final result is 12 ppb, the final result is 12 x 1.5 = 18 ppb.

12.4 TEST PROCEDURES

a. Sample Analysis.

(1) Allow reagents, microwells, and sample extracts to reach room temperature prior to running the test.

(2) Insert a sufficient number of wells into the microwell holder for all standards and samples to be tested. (For example: to test 7 samples use 8 wells - 1 for the standard and 7 for the test samples).

Test Strip

Well #	1	2	3	4	5	6	7	8
Sample	C 0	S1	S2	S3	S4	S5	S6	S7

Where C 0 is the zero control, S1 is sample 1, S2 is sample 2, S3 is sample 3, etc.

NOTE: Do not run more than 3 strips (23 samples) per set of control standards.

(3) Using a new pipette tip for each standard and sample, pipet 50 µl of standard and prepared sample to separate wells.

(4) Add 50 µl of enzyme conjugate (red capped bottle) into each well.

(5) Add 50 µl of anti-aflatoxin antibody (black capped bottle) into each well.

(6) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(7) Incubate for 10 minutes (± 1.0 minutes) at room temperature (64 – 86º F).

(8) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.

(9) Using a wash bottle, fill each well with distilled or deionized water or washing buffer solution. Empty the wells again and remove all remaining liquid. Repeat this step 2 times (total of 3 washes).

(10) Add 100 µl of substrate/chromagen (white dropper bottle) to each well.

(11) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(12) Incubate for 5 minutes (± 0.5 minutes) at room temperature (64 – 86º F). Cover the wells with a paper towel to protect them from light sources.

(13) Add 100 µl of stop solution (yellow or orange dropper bottle) to each well.

(14) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(15) Measure absorbance at 450 nm using the Biotek EL 301, or Awareness Technology Stat-Fax Model 303 PLUS microwell readers.

(Results must be read within 10 minutes)

b. Reading Results with the Microwell Reader.

(1) Biotek EL 301 Microwell Reader.

(a) Make sure that the microwell reader is on and allowed to warm-up for a minimum of 15 minutes before using.

(b) Remove sample carriage and hit "Enter."

(c) Insert W2 filter and hit "Enter."

(d) Insert W1 filter (450 nm) and hit "Enter."

(e) Hit "Clear" and then "Blank." This will cause the instrument to read air as the blank sample.

(f) Load microwells into sample carriage so that the first control labeled 0 is in position A1.

(g) Load the sample carriage into the strip reader so that position A1 is under the light beam of the reader.

(h) Press "Read" and an absorbance value for A1 should appear in the display on the microwell reader. Record the value.

(i) Slide the carriage to position A2 and press "Read." An absorbance value for A2 will appear. Record the value.

(j) Repeat step (i) until absorbance values have been obtained for the control and all samples. Record the values.

(k) Use the RIDA®SOFT Win Data software provided by r-Biopharm to convert the absorbance values into concentration values. **Remember to multiply results for DDGS by 1.5.**

(2) Stat-Fax Model 303 PLUS Microwell Reader

(a) To begin from the "Ready" prompt, press Menu, key in the test number, and then press Enter.

(b) The screen will read, "Set carrier to A, press enter." Place the wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, then press enter. The carrier will advance into the reader, and it should start to print.

(c) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N?"

Press "Yes" (1/A) to print the graph,

Press "No" (0) to skip this feature.

(d) The screen will read, "Accept Curve Y/N ?"

Press "Yes" (1/A) to accept the curve and proceed to read another strip. When finished reading the second strip, press "Clear" twice and the results strip will print, "Test Ended."

Press "No" (0) to end the test. **Remember to multiply results for DDGS by 1.5.**

12.5 REPORTING AND CERTIFYING TEST RESULTS

a. Report all results on the pan ticket and the inspection log to the nearest whole ppb.

b. Sample results over 100 ppb are reported as >100 ppb unless a supplemental analysis is performed.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

12.6 SUPPLEMENTAL ANALYSIS

a. Diluting the Sample Extract.

If quantitative results are above the testing limits (i.e., 100 ppb) of the test kit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 100 ppb, the sample extract must be diluted so that a value between 5 and 100 ppb is obtained.

The final aflatoxin concentration is calculated by multiplying the results with the diluted extract by the dilution factor.

b. Example.

If the original analysis reported the aflatoxin value at greater than 100 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

(1) Prepare a 35% methanol dilution solvent by adding equal portions of distilled or deionized water and 70/30 methanol/water extraction solvent. Example: 10 ml of water + 10 ml of 70/30 methanol mixture.

(2) Dilute 200 µl (0.2 ml) of the diluted extract (obtained from of the original extract (obtained from section 12.3, step a (5), or b (6), as applicable) with 1.8 ml of the dilution solvent mixture from step 1 above. The total volume is 2 ml. This is a 1 to 10 dilution (compares volume in the beginning with the total volume in the end).

(3) Proceed to sample analysis.

(4) Multiply the analytical results obtained by 10 to obtain the actual aflatoxin concentration. For example, if 25 ppb was the original value obtained with the diluted extract, the actual concentration in the original sample was 250 ppb.

12.7 CLEANING LABWARE

a. Negative Tests (≤ 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour the liquid down the drain and place the materials in a garbage bag and discard.

12.8 WASTE DISPOSAL

a. Negative Results (≤ 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the syringe into a plastic garbage bag for disposal.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the remaining ground portion must be decontaminated, using bleach, prior to disposal. Discard the filter syringe and remaining ground portion into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

12.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits

(1) 1 microtiter plate.

(2) 48 antibody coated microwells.

(3) 1 aflatoxin standard solution of 1.3 ml of 0 ppb aflatoxins.

(4) 1 red-capped bottle of 3 ml peroxidase conjugated aflatoxin solution.

(5) 1 black-capped bottle of 3 ml anti-aflatoxin antibody.

(6) 1 white dropper bottle of 6 ml Substrate/Chromagen.

(7) 1 yellow or orange dropper bottle of Stop reagent.

(8) 1 washing buffer.

b. Materials Required but not Provided:

(1) Stabilization Buffer (for analysis of DDGS) (P4341)

(2) Methanol - ACS grade or better.

(3) Deionized or Distilled Water.

(4) 250 ml graduated cylinder.

(5) 125 ml container.

(6) Filtering syringe (JM1000), Whatman No. 1 filter paper, or equivalent.

(7) Sample collection tubes.

(8) Waring high-speed blender with a one liter jar, or equivalent.

(9) Sample grinder.

(10) Balance.

(11) Biotek EL 301 or an Awareness Technology Inc. Stat-Fax Model 303 Plus Microwell reader equipped with a 450-nm filter.

(12) Eppendorf Repipettor, or equivalent, and 2.5 ml syringes.

(13) 50 µl, 100 µl, and 1000 µl pipettor and pipette tips.

(14) Paper towels, Kaydry paper or equivalent absorbent material.

(15) Waste receptacle.

(16) Timer: 3 channel minimum.

(17) Waterproof marker, Sharpie or equivalent.

(18) Wash bottle.

12.10 STORAGE CONDITIONS

a. Storage Conditions.

(1) The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 35° F and 46° F. **(DO NOT FREEZE)**

(2) Return any unused microwells to their original foil bag and reseal them together with the desiccant provided.

(3) The substrate/chromogen solution is light sensitive, therefore, avoid exposure to direct light.

b. Indication of Instability or Deterioration of Reagents.

(1) Any bluish coloration of the red stained substrate/chromogen solution is indicative for deterioration and the reagent should be discarded.

(2) A value of less than 0.6 absorbance units for the zero standard may indicate deterioration of reagents.